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RESEARCH ARTICLE

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The effects of bedding materials on learning and memory performance and texture preference in rats

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ABSTRACT

The present study was designed to investigate the effect of different available bedding materials on learning and memory performance, bedding texture preference as well as intra-cage ammonia concentration in rats. The animals were housed on different bedding types for two weeks. Bedding materials were produced in the same sizes from poplar, walnut, pistachio, apricot, almond woods and alfalfa steam and live. Spatial and passive avoidance learning and memory were assessed by Morris water maze (MWM) and shuttle box tasks. A modifying six-arm radial maze was used to assess bedding texture preference by rats. For each bedding groups, average ammonia level (ppm) over a week was calculated. The data indicated that the rats that had walnut and almond chips show better learning and memory performance in both MWM and shuttle box tests than other groups. The weakest learning and memory performances were observed in rats exposed to alfalfa bedding. In texture preference test, the rats spent more time in walnut and almond arms, and less time in alfalfa. Besides, the total water and food intake as well as the number of visit to alfalfa arm were decreased as compared to other arms. Moreover, in alfalfa bedding cage, average intra-cage ammonia level was utmost. Overall, current bedding materials may contain diverse biochemically effective compounds or individual micro edges which alter learning and memory performances of rats.

Keywords

Bedding materials, Learning and memory, Texture preference, Rats

Abbreviations

MWM: Morris water maze
STL: Step-through latency
TDC: Time spent in the dark compartment
LTP: Long term potentiation

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Introduction

The normal physiological functions of laboratory animals were strongly affected by their housing and husbandry conditions. In particular, bedding is one of the most important housing elements, which has influence on various neurobiological functions of laboratory animals [1-3]. Ethogram of mice behaviors including agonistic interaction, feeding, drinking, locomotion, nest-building and resting show bedding material-related changes [4]. On behalf of rodents, various kind of wood shavings such as paper, corncobs and hill rice are known as common bedding substances. There is no definition for ideal bedding, however, ideal bedding must have a low ability of infection, high absorbency, low allergenic activity, minimal chemical toxicity, low cost and high accessibility and compatibility [4-7].

It has been indicated that variations of bedding materials affects the stress and immune system [8], somatosensory signaling [9], neuropathic pain[10], vocalizations [11], body mass [12], temperature regulation, metabolism [13, 14], as well as liver enzyme levels in laboratory rodents [15, 16]. In particular, rodent's neurocognitive development has been affected by bedding materials and housing conditions. It has been indicated that corncob bedding suppresses estrogen-dependent aggressive behavior in rats [17]. In another study, mice housed in cages containing pulp chips, for 8 week, show better water maze performance than wood flakes group [18]. It has been indicated that exposure to an enriched environment induces dendritic branching and synaptogenesis in cortex and hippocampus of rats. Besides, housing enrichment can improve learning, memory, and synaptic plasticity in rodents[19]. Housing rats in cages with limited nesting/bedding materials impaired spatial learning and memory and hippocampal long term potentiation (LTP). Providing enriched environment can overcome the memory impairments through the recovery of LTP [1].

Preference testing has indicated that rodents have different preferences for bedding substances. Additionally, another study on rats and mice indicated that animals had strong preference for large fibrous bedding particles than relatively small particles [20]. It has been also reported that mice prefer cloth bedding type in comparison to wood shavings, paper and polycarbonate bedding substances [21]. Bedding material preferences for animals are determined by various chemical and physical features including color, odor, chemical compounds, and texture properties such as condition of surfaces, edges and coarseness.

In the present study, bedding texture preference was evaluated by housing the rats on six different bedding types including standard hard bed poplar chip, dried alfalfa as typical soft bedding material; walnut, apricot and almond as unusual bedding, and pistachio chip as very exclusive hard bed available in Iran and a few countries. Regarding climate change in Iran many large quantities of pistachio, walnut, apricot, and almond at low cost are available and can be used as bedding surface. We also examined bedding material mediated possible alterations in learning and memory performance of rats. In each bedding cage, ammonia concentration was also assessed.

Results

MWM test

There was a significant difference in the escape latency time among experimental groups during acquisition blocks [F (3,480) = 44.52, $p = 0.001$]. In the first day, the latency time to find the hidden platform was significantly decreased in the rats housed on walnut and almond as compared to other groups ($p < 0.05$). In the second day, there was a significant decrease in the escape latency of rats housed on almond as compared to apricot and poplar groups ($p < 0.05$) (Fig. 1, upper graph). In addition, the main escape latency was significantly different among groups [F (5,165) = 15.93, $p = 0.001$]. The rats housed on walnut and almond bedding showed the lowest latency time to find the hidden platform (Fig. 1, lower graph).

Significant differences were observed in the travelled distance to find hidden platform among experimental groups during acquisition days [F (3,480) = 31.47, $p = 0.001$]. In the first day, the rats housed on walnut and almond travelled lower distance to find the hidden platform as compared to rats that were housed on poplar and pistachio ($p < 0.05$). In the second day, rats kept on almond travelled lower distance to find the hidden platform in comparison with pistachio, apricot and alfalfa group ($p < 0.05$). In the third day, rats kept on almond and poplar travelled lower distance to find the hidden platform as compared to walnut, pistachio, apricot and alfalfa group ($p < 0.05$) (Fig. 2, upper graph). In the fourth day, however, rats subjected to walnut travelled lower distance to reach the hidden platform as compared to alfalfa and pistachio groups ($p < 0.05$). Furthermore, the main travelled distance to find the platform was significantly decreased in rats reserved on almond ($p < 0.05$) and walnut bedding ($p < 0.05$) in comparison to pistachio, alfalfa, and apricot (Fig. 2, lower graph).

The results of probe test indicated that time spent in the target zone was significantly increased in rats kept on walnut and almond as compared to poplar, apricot and alfalfa groups ($p < 0.01$) (Fig. 3).

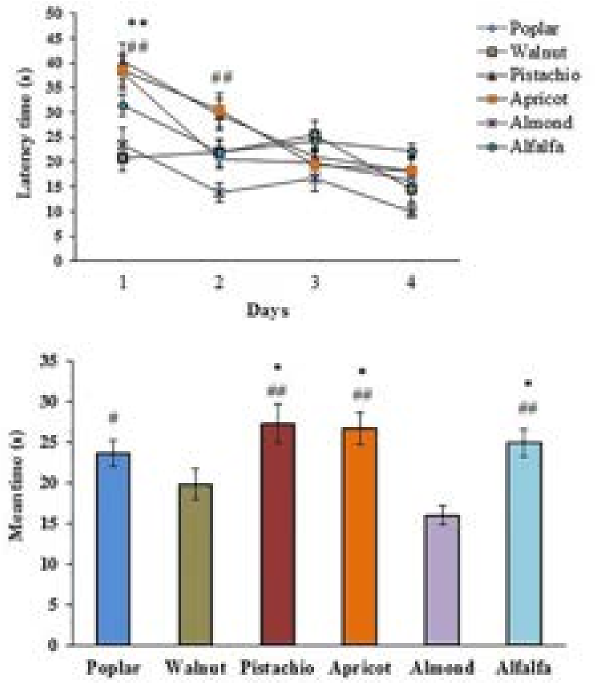

Figure 1.
Evaluation of the escape latency in each acquisition block (upper graph) and the mean scape latency (lower graph) in rats subjected to different bedding surfaces.
*: $p < 0.05$, **: $p < 0.01$ vs walnut group
#: $p < 0.05$, ##: $p < 0.01$ vs almond group

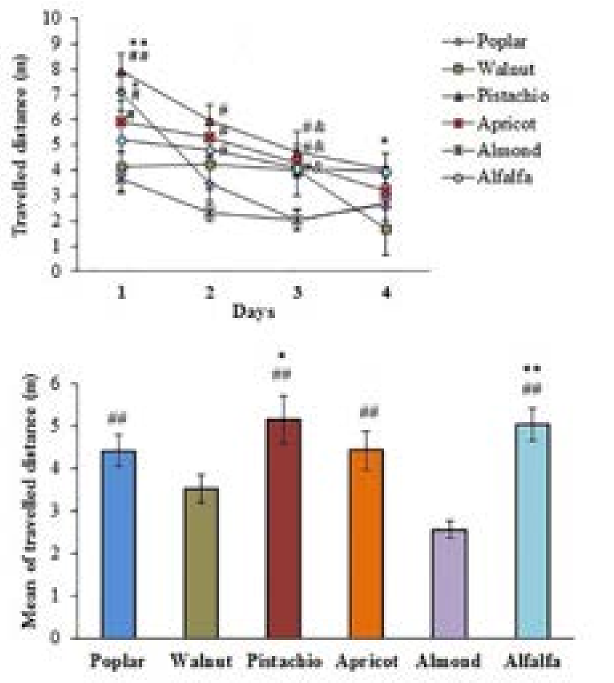

Figure 2.
Evaluation of the travelled distance in each acquisition block (upper graph) and the mean travelled distance (lower graph) in rats subjected to different bedding surfaces. Data are presented as mean ± SEM.
*: $p < 0.05$, **: $p < 0.01$ vs walnut
##: $p < 0.01$, #: $p < 0.05$ vs almond
&: $p < 0.01$ vs poplar.

Shuttle box test

As shown in Fig. 4A, the number of trials required to reach acquisition were significantly decreased in walnut, apricot and almond groups in comparison with poplar group ($p < 0.05$). In the retention test, rats housed in alfalfa bedding substance showed significant decrease in STL ($p < 0.05$) and increase in TDC ($p < 0.001$) as compared to other groups (Fig. 4B and 4C).

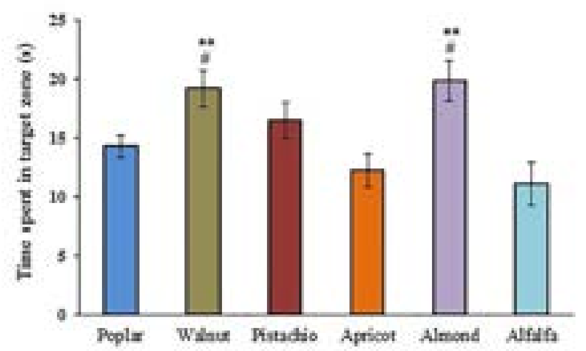

Figure 3.
Evaluation of the time spent in the target zone in probe test between the groups of rat subjected to the different bedding surfaces. Data are presented as mean ± SEM.
**: $p < 0.01$ vs apricot and alfalfa
#: $p < 0.05$ vs poplar

Ammonia concentration

Intra-cage ammonia level was recorded during the first week of experiment. As shown in Fig. 5, the mean ammonia concentration in alfalfa cage was significantly increased in comparison with others groups ($p < 0.001$).

Bedding preference

During the first week, the rats spent highest time in walnut and almond ($p < 0.001$) and lowest time in the alfalfa comprised arms ($p < 0.001$) (Fig. 6A). In addition, the rats had more visit to arms containing almond than pistachio and apricot ($p < 0.05$) and alfalfa ($p < 0.001$). Besides, rats visit into the cage containing alfalfa bedding was lowest (Fig. 6B). Moreover, As shown in Fig. 7A, the total water consumption by rats were significantly increased in walnut, almond as well as poplar cages in comparison with pistachio and alfalfa cages ($p < 0.01$). In addition, the total food consumption was significantly increased in almond cage as compared to pistachio and apricot ($p <0.05$) as well as alfalfa cage ($p < 0.01$). Moreover, food consumption

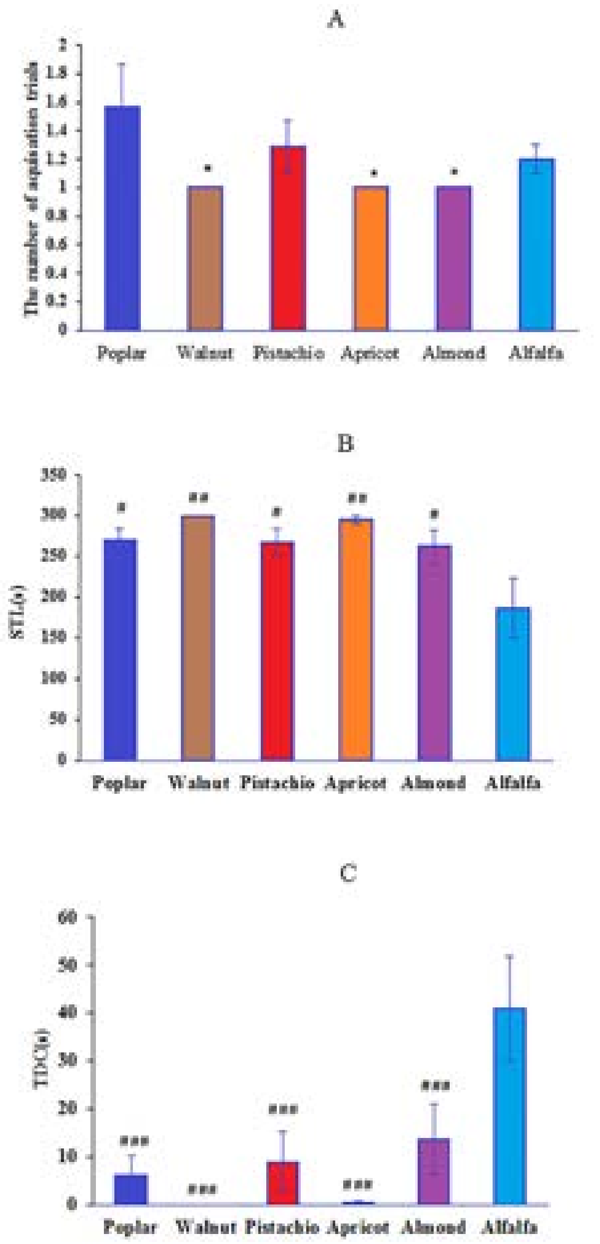

Figure 4.
Evaluation of the number of acquisition trials to reach successful learning (A), the step through latency (STL) (B) and time spent in dark chamber (TDC) (C) in shuttle box test in the groups of rats subjected to different bedding types. Data are presented as mean ± SEM.
*: $p < 0.05$ vs poplar group
#: $p < 0.05$, ###: $p < 0.001$ vs alfalfa

was significantly increased in poplar and almond cages in comparison with alfalfa ($p < 0.05$) (Fig. 6B).

Discussion

The present study investigated the learning and memory performances of rats subjected to different bedding chips including poplar, walnut, pistachio, apricot, almond, and alfalfa. According to the results, in comparison with other groups, rats housed on walnut and almond chips showed better learning and memory performance in MWM and shuttle box tests. The learning and memory performances of rats subjected to alfalfa bedding were lesser than other bedding types. Besides, investigation preferences of rats for types of bedding by a six-arm radial maze showed that rats spent more time in chips of walnut in comparison with pistachio, apricot and alfalfa bedding types. In addition, the rats had highest and lowest visit into almond and alfalfa-containing cage, respectively. Likewise, food and water intake by rats in alfalfa contain cage was lowest. There are only a few studies on the substance quality as bedding material. The present study, tried to fill the gap of data about pistachio, apricot and almond.

It has been well documented that bedding and husbandry have influence on rodent's neurophysiologic responses. However, just a few studies have shown bedding texture property on cognitive-related behaviors. A study by Tanaka and colleagues showed that mice subjected to pulp chips bedding, over eight weeks period, had better water maze performance than those kept on wood flakes [18].

The results of this study also showed that the rats find walnut and almond sawdust more suitable as a resting surface than other four bedding types. It shows direct relationship between comfortable bedding texture and learning and memory performances. The bedding materials used here due to their structure have diverse edge and surface which may result in changed stimulation of the plantar foot area [22]. In other words, edge of the bedding show different mechanical effect on rat planter surface. It has been indicated that the daily behaviors of rats such as nesting and sleeping are affected by kind of bedding materials [10]. Besides, bedding material features may affect sensory processes even down to the molecular and cellular levels [10].

Here, the rats subjected to walnut and almond chips showed better learning and memory performance. Nuts, leaves, woods and hulls from walnut and almond are highly valued for their biological properties. In particular, the compounds have potential health-promoting activities because of their phenolic-enriched contents. It has been indicated that walnut polyphenol improves learning and memory performances of hypercholesterolemia mice [23]. It has been indicated that flavonoid and other polyphenols material improve learning and memory performances mainly via their antioxidant capacity and cholinesterase activities [24-26]. In the present study, it is likely that beddings different influences on learning and memory are partially mediated by differences in their polyphenolic capacity. However, we did not explore polyphenols capacity of different chips and to clarify

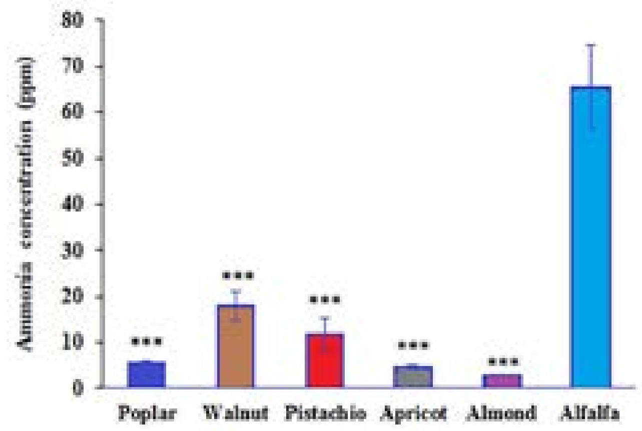

Figure 5.
The average of ammonia concentration in each bedding cage. Data are presented as mean ± SEM.
***: $p < 0.001$, vs alfalfa

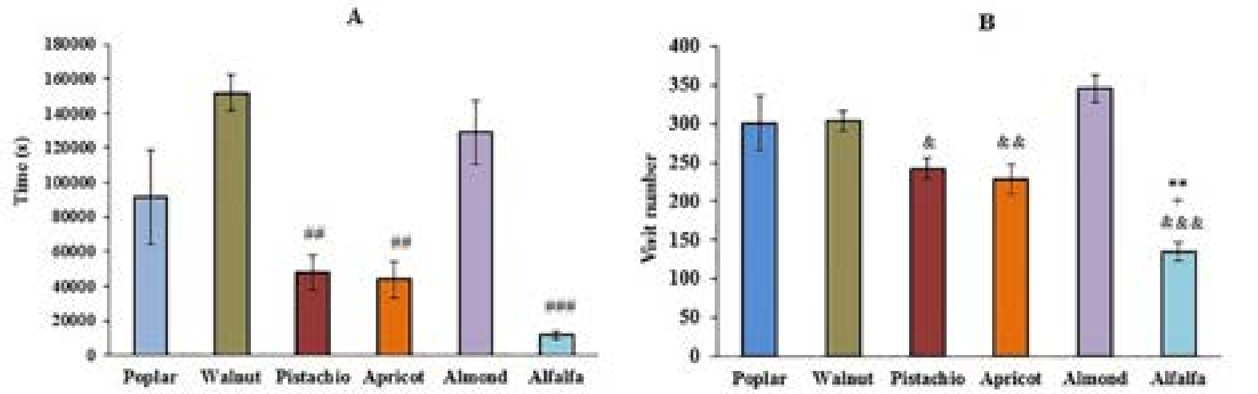

Figure 6.
Assessment time spent and the number of visits by rats into each arm of radial maze containing different bedding substances during a week. Data are presented as mean ± SEM.
**: $p < 0.01$ vs poplar and walnut
##: $p < 0.05$, ###: $p < 0.001$ vs walnut and almond
+: $p < 0.05$ vs pistachio and apricot
&: $p < 0.05$, &&: $p < 0.01$, &&&: $p < 0.001$ vs almond

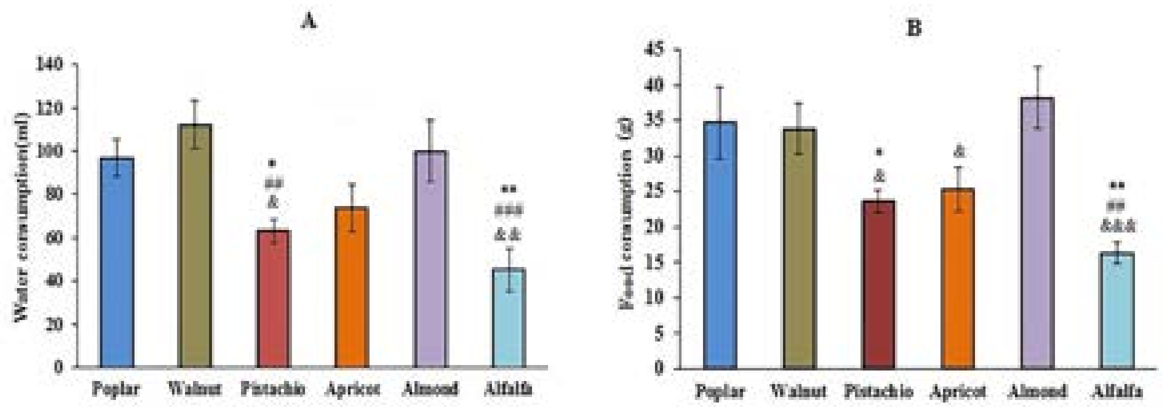

Figure 7.
Comparison of food and water consumption by rats in the maze containing different bedding substance in each arm. Data are presented as mean ± SEM.
*: $p < 0.05$, **: $p < 0.01$, vs poplar
##: $p < 0.05$, ###: $p < 0.001$ vs walnut
&: $p < 0.05$, &&: $p < 0.01$, &&&: $p < 0.001$ vs almond

such relationship further studies are still required.

To the best of our knowledge the study was the first to consider association between bedding preference and learning and memory related behaviors. However, some studies have indicated that improvement of nesting conditions positively modulate learning and memory -related functions. It has been demonstrated that environmental enrichment improves learning and memory in various laboratory tasks [2]. In addition, environmental enrichment can induce morphological changes in the cortex and hippocampus of rat such as enhancement of neuron numbers, synapses and dendritic branches [3, 27]. Increasing bedding volume has been correlated with intracage ammonia decrease [4, 28]. Moreover, there is significant relationship between the depth of bedding and animal preference as well as animal physiology [4].

Welfare of laboratory animals are closely related to optimum intracage features like the level of ammonia, moisture, and absorbency and bacterial growth [29]. In the current study, assessment of ammonia concentration in each cage showed that there was the highest intracage ammonia in the cage containing alfalfa bedding. As shown in the results, the mean ammonia level was lower than adverse level 100 ppm or 130 ppm [22, 30]. Metabolized urea from urine and feces of the animal is responsible for ammonia production [31].

Interestingly, the rats housed on alfalfa bedding surface showed lowest learning and memory performance. Various studies have been reported on the distractive effect of ammonia on brain functions. Increased ammonia concentration in the brain as a result of diseases could induce a range of neurobehavioral dysfunctions like learning and memory deficient, sleep-wake inversions, brain edema and seizures [32, 33]. It has also been reported that inhalation of ammonia was able to reduce cognitive performance for culture fair, digit symbol and vocabulary[34]. Besides, increased intra cage CO_2 levels and fecal cortisol concentrations have crucial role in animal function and behaviors [4], but were not included in this study.

Conclusion

This study provides some primary data supporting the relationship between bedding texture preference and learning and memory function of rats. The effect may be partially mediated by bedding differences in ammonia absorption capacity. It also could be related to biochemical diversity and individual micro edges of distinct bedding materials.

Material and methods

Animal and housing conditions

Adult male Wistar rats (230–270 gr) were used. The animals were housed in conventional animal room (3× 8× 3 m) under a 12 h light/dark cycle in controlled condition with temperature of 22 ± 2 °C. The ventilation rate was 8-15 times per hour. During the experiment the same amount of food and water was available ad libitum. All experimental procedures were approved by the Animal Research Ethics Committee of the Shahid Bahonar University of Kerman, Iran.

Experimental design

Experiment 1: Evaluation of the spatial and passive avoidance learning and memory performances

The rats were divided into six experimental groups and kept on the same amount of different bedding substances for 2 weeks. Bedding materials included were chip of poplar, walnut, pistachio, apricot, almond and dried alfalfa. The wood ship average particle sizes were 15 × 4 × 1 mm with a moisture of 8%, which was sieved (purchased from zist mehvar-pajoheshe Pars Company, Kerman, Iran). The number of animals per group and per cage were seven. The amounts of animal biomass per cage were the same in the beginning and during the study. The rats learning and memory performances were evaluated using Morris water maze (MWM) and shuttle box tests. Besides, during first week of the experiment daily changes in intracage ammonia concentration was measured using multigas transmitter (ModBus, TM-1280). To take a reading, the sampling tube was inserted in the middle of bedding. The monitor took continuous reading for 5 min, for measuring ammonia level according manufacturer's recommendations. The experimenters were not blinded to the bedding types; they had no expectations of any group differences.

Experiment 2: bedding material preference

Bedding texture preference by rats (n=7) was assessed using a modified bedding preference test system introduced by Blom [20]. Briefly, the maze consisted of a central wire mesh circle area surrounded by six enclosed wooden cages (50 × 10 × 40 cm). The central zone was raised 2 cm above the floor than the cages. Equal amounts of six bedding materials, almost the same in size and shape, were situated on different cages of the maze. In each cage food and water was ad libitum. For beginning the experiment, each rat was placed on the central area of maze and during one-week period the rats' behavior including the number of visit and time spent in each arm were monitored by a video camera system. Besides, rats' food and water intakes in different cages of maze were measured. Before this test, rats were housed on pine shavings.

Assessment of learning and memory performance

Shuttle box test

PA learning and memory was assessed by a shuttle box apparatus as previously described. The test protocol was divided into a learning session on the first day and a test trial 24 h later. For the learning trial, each animal was placed in light chamber of shuttle box apparatus. Then, the door was opened and the animal was allowed to enter into the dark sector. The animal received an electrical shock (0.5 mA, 50 Hz, 2 s once) upon entrance to dark sector via the stainless steel floor. The learning trial was terminated when the rat remained in the light chamber for 5 consecutive minutes. After one day, the retention test for assessing memory, each rat was placed in the light compartment of shuttle box device. After 30

seconds, the door was opened and the step-through latency (STL), the time before the first entry of the rat to the dark sector, and total time spent in the dark compartment (TDC) were recorded.

Morris water maze (MWM) test

Spatial learning and memory was assessed by MWM pool. It consisted of a dark circular pool (136 cm in diameter and 60 cm high) filled with water (20 ± 1 °C) to a depth of 25 cm. The extra maze cues were placed in consistent locations on the walls which were visible to the rats. The pool was divided into four quadrants defined by the four cardinal directions. A circular platform was located 2 cm below the water surface in the middle of one of the quadrants. At the beginning of experiment, each rat was lightly placed in the water facing the wall of the pool from one of the directions. The location of each rat was tracked by a digital TV system and analyzed using the Ethovision video tracking system (Noldus Information Technology, the Netherlands). One day prior to the beginning of training; the rats were habituated to the pool by allowing them to swim for 60 s without the platform.

The test included acquisition and probe trials. The acquisition test was performed on 4 consecutive days with four trials per day with a 5 min interval between trials. The rats were allowed to swim within 60 s to find the hidden platform at each trial. Once the platform was found, the animal would have to stay on the platform for 30 s. If unsuccessful within 60 s, it was gently guided to the platform for 30 s. The escape latency, traveled distance and swimming speed for each rat were evaluated. In probe test, one day after acquisition test, the hidden platform was removed from the pool and rats were placed in the quadrant opposite the target quadrant and allowed to swim freely for 60 s. The time spent in the target quadrant was recorded and analyzed as a measure of spatial memory retention.

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). The acquisition test data related to four acquisition days of MWM test were analyzed using repeated measures ANOVA. The statistical significances for probe and preference tests were determined by one way ANOVA followed by post-hoc Tukey's test. The results of shuttle box test were analyzed by Friedman and Kruskal-Wallis tests followed by Mann-Whitney U test.

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Author Contributions

M.A and S.EM designed the study; A.T and R.N collected data; R.K analyzed the data and wrote the paper.

Conflict of Interest

None .

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